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Human T-Cell Lymphotropic Virus Type-I Infection in the Severe Combined Immunodeficiency Mouse

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Human T-cell lymphotropic virus type-I (HTLV-I) is the etiologic agent of HTLV-I-associated myelopathy/tropical spastic paraparesis (HAM/TSP) and adult T-cell leukemia (ATL). HAM/TSP and ATL occur infrequently among HTLV-I-infected individuals, and rarely develop in the same individual. To study host and viral factors involved in the induction, tissue tropism, as well as pathogenesis of HAM/TSP, peripheral blood lymphocytes (PBL) from 14 patients with HAM/TSP and from 9 controls were introduced into severe combined immunodeficiency (SCID) mice by intraperitoneal injection. Mice were followed for up to 26 weeks. Human IgG was produced from 2 to 14 weeks after reconstitution in all animals. Thirty-two of 44 mice (72%) showed circulating human antibody against the major viral protein products of HTLV-I. Analysis of viral sequences by polymerase chain reaction (PCR) demonstrated HTLV-I sequences in 21/38 (55%) brains and in 7/17 (41%) spinal cords from HTLV-I-hu SCID mice. No animal had clinical evidence of neurological impairment or pathological findings similar to those seen in HAM/TSP. Seven mice who received PBL from Epstein Barr virus (EBV)-seropositive patients developed an intraperitoneal lymphoma. In 2 mice an infiltration of brain by a lymphoblastic tumor of B/T cell type was observed. By PCR, all the tumors were EBV-positive; HTLV-I sequences were detected in 5 of them. Our study suggests that the HTLV-I-hu-SCID mouse provides a potentially valuable system for studying the production, kinetics, and pathogenicity of anti-HTLV-I antibody, and may help clarify the interaction of EBV and retroviruses in the development of disease.

KEY WORDS: SCID mouse, HTLV-I, HAM/TSP, ATL, EBV, tumorigenesis

INTRODUCTION

The severe combined immunodeficiency (SCID) mouse provides a suitable environment for the study of human lymphoid cell xenografts [McCune et al., 1988; Mosier et al., 1988]. When injected into the peritoneum of SCID mice (hu-SCID), human peripheral blood lymphocytes (PBL) synthesize human antibodies that circulate in the mouse to a level comparable to that seen in humans without major graft-versus-host reaction [Martino et al., 1993a]. For these reasons, the hu-SCID mouse model has been used for investigations of autoimmune [Davies et al., 1991; Duchosal et al., 1990; Krams et al., 1989; Martino et al., 1993b; Tighe et al., 1990] and viral [Cannon et al., 1990; Namikawa et al., 1988] human diseases.

Human T-cell lymphotropic virus type I (HTLV-I) is the etiologic agent of HTLV-I-associated myelopathy/tropical spastic paraparesis (HAM/TSP) and adult T-cell leukemia (ATL) [Höllsberg and Hafler, 1993; Iwasaki, 1993]. Even in areas endemic for HTLV-I infection the occurrence of HAM/TSP and ATL is relatively infrequent and co-morbidity is rare [Kawai et al., 1989]. Factors affecting disease induction and HTLV-I tissue tropism are still unknown, partly because of the absence of an adequate animal model reproducing the immunological and pathological features of the human disease.

A model is described of HTLV-I infection established by transplantation of PBL from HAM/TSP patients into the peritoneal cavity of SCID mice.

PATIENTS AND METHODS

Patients

PBL were obtained from 14 patients (12 women, 2 men) with a mean age of 48.6 years (range 30–67 years) affected by HAM/TSP (disease duration 7.0 years; range

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2–11 years). The diagnosis of HAM/TSP was made according to criteria of the World Health Organization [Osame, 1990]. No patient was receiving steroids or other immunosuppressive treatments at the time the samples were obtained.

Mice and Cell Transfer

C.B.-17 scid/scid mice (Harlan Sprague Dawley, Indianapolis, IN) were maintained in a pathogen-free environment. In order to avoid unsuccessful transplantation due to animal phenotypic "leakiness" [Bosma et al., 1988], we did not attempt reconstitution in mice containing mouse serum IgM > 2 μg/ml as determined by enzyme linked immunosorbent assay (ELISA) [Martino et al., 1993a].

PBL isolated from each of the 14 HAM/TSP donors were inoculated into 4–6 SCID mice. Controls included two naive SCID mice, 7 SCID mice transplanted with PBL from 2 healthy subjects, and 7 SCID mice transplanted with PBL from 4 patients with multiple sclerosis. PBL from HAM/TSP patients and controls were obtained by Ficoll density gradient separation. SCID mice received from 15×10^6 to 14×10^7 PBL diluted in 1ml of Hanks' balanced salt solution and were introduced by intraperitoneal (i.p.) injection using a 25 gauge needle. Animals were bled every two weeks from the tail vein and killed between 2 and 22 weeks after transplantation (a.t.). Transplantation was considered successful when the level of circulating human IgG at 2 weeks a.t. in the mouse was greater than 10 μg/ml.

Antibody Analysis

Human IgG was measured by an ELISA using affinity purified rabbit anti-human IgG or IgM followed by affinity purified rabbit phosphatase conjugated anti-human IgG or IgM [Martino et al., 1993a].

The presence of anti HTLV-I human antibody was tested by Western blot (Diagnostic Biotechnology Ltd., Singapore) in serum samples diluted 1:50 in phosphate buffered saline. Immunoreactive bands were visualized on nitrocellulose strips by the alkaline phosphatase reaction.

Neuropathological Analysis

After death, mouse brains and spinal cords were divided in two. One part was fixed in 4% paraformaldehyde and the other immediately frozen. Paraformaldehyde-fixed tissues were paraffin-embedded or isopentane-frozen, cut into 7-μ-thick sections, and stained with hematoxylin and eosin (H & E) or by the Kluver-Barrera method and Luxol fast blue for myelin staining. The phenotype of human lymphoid cells, visualized after H & E staining, was evaluated by indirect immunostaining of serial sections using anti-CD20 antibody and anti-CD3 or anti-CD45RO+ (DAKO S.p.A., Milano, Italy). Signal amplification was obtained using streptavidin-biotin immunoperoxidase and diaminobenzidine (DPC, Los Angeles, CA). Immunolabelled sections were counterstained with hematoxylin.

PCR Studies

To demonstrate the presence of HTLV-I DNA sequences in transplanted SCID mice, polymerase chain reaction (PCR) was performed on DNA extracted from snap-frozen brain, spinal cord, spleen, peritoneal fluid cells, liver, lung, heart, kidney, peripheral blood, and tumors (when present) of 39 HTLV-I hu-SCID mice and 2 uninoculated control animals. In some cases primers of the long terminal repeat (LTR) region were used [AAC TCC TGC ATT TTT TCT TTC CTA GC (5'primer); AAA AGA GCG GGA GAA AGA GGA GG (3'primer)] for PCR amplification, followed by Southern blot hybridization with an internal probe (CTC CTA GCG ACG TCA GCG GCC) radiolabelled with 32P by the T4 polynucleotide kinase (New England Biolabs, Beverly, MA). The reaction (30 cycles at 94°C, 55°C, and 72°C) was performed with 1 μg of template DNA in the presence of 2.5 mM $MgCl_2$. In other cases, a nested PCR was performed using external and internal primers in the pX region of the viral genome followed by analysis of the amplification products on 5% sodium dodecyl sulfate polyacrylamide gels [Matsumoto et al., 1990]. One μg of DNA extracted from an MT4 HTLV-I infected cell line was used as positive control.

The presence of Epstein Barr virus (EBV) DNA was assessed by nested PCR [Cinque et al., 1993]. The amplification product was analyzed by agarose gels electrophoresis followed by ethidium bromide staining.

RESULTS

From 100 to 10,000 μg/ml of circulating human IgG were detected from 2 up to 8 weeks a.t. in the 14 transplanted mice we tested. This result confirmed the successful transplantation of the human PBL.

Anti-HTLV-I antibodies were found by Western blot in the serum of 32 of 44 (72%) HTLV-I hu SCID mice. At 4 weeks a.t., all positive murine serum samples showed a pattern of viral antigen recognition similar to that of the corresponding human donor (Fig. 1), although HTLV-I hu-SCID mouse serum samples occasionally recognized different viral antigens. Anti-HTLV-I antibody disappeared from mouse sera approximately 12 weeks a.t. (Fig. 2) probably as a result of the death of most antibody-producing human cells [Martino et al., 1993a].

HTLV-I DNA genome was detected by PCR in several HTLV-I hu-SCID mouse tissues. No consistent pattern of virus detection was observed from mouse to mouse among the various tissues amplified (Fig. 3). The pattern and frequency of HTLV-I provirus distribution varied even among mice reconstituted with PBL from the same human donor. Amplification of viral sequences was achieved in 21/38 (55%) of mouse brains, 7/17 (41%) spinal cords, 14/23 (60%) peritoneal fluid cells, 20/34 (58%) spleens, 27/38 (71%) livers, 15/28 (53%) PBL, 16/30 (53%) lungs, 18/27 (66%) hearts, and 12/28 (42%) kidneys.

None of the animals showed evidence of neurological dysfunction up to 26 weeks a.t. None of the pathological alterations typical of HAM/TSP (including myelin loss)

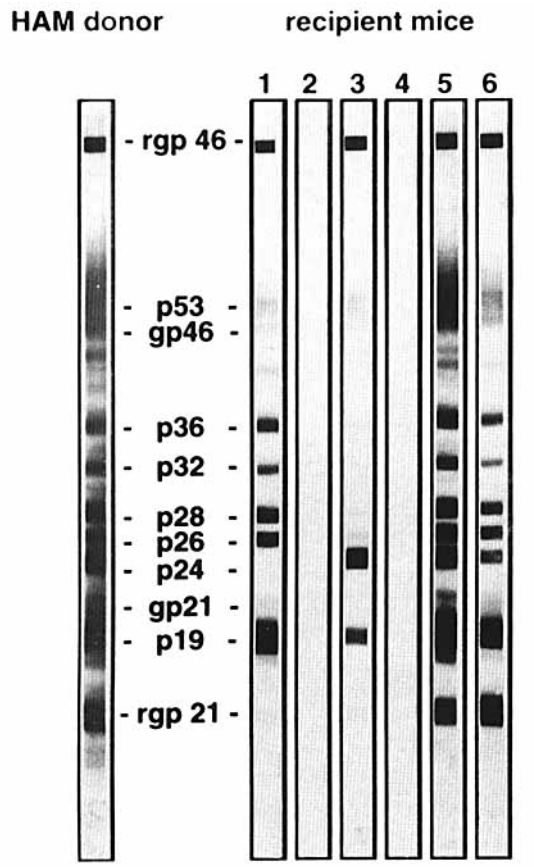

Fig. 1. Western blot analysis of anti HTLV-I antibody reactivity of HTLV-I hu-SCID sera compared to the serum of the corresponding human donor. **Lanes 1–6** represent sera from 6 different HTLV-I hu-SCID mice transplanted with PBL from a single HAM/TSP patient. Seropositivity was observed in 4 serum samples (**lanes 1, 3, 5,** and **6**). The serum from one HTLV-I hu-SCID mouse **(lane 5)** shows a pattern of antibody reactivity identical to that of the HAM/TSP donor **(unnumbered left-ended lane).** Serum samples were obtained at 4 weeks a.t. from all animals.

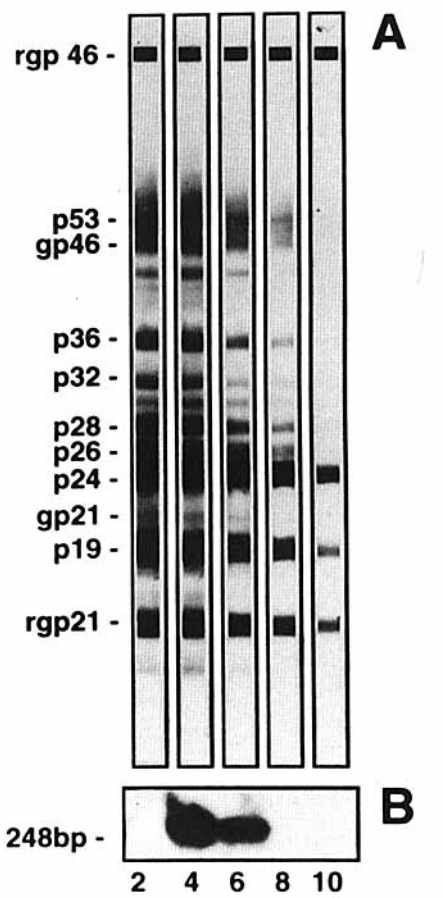

Fig. 2. **A:** Kinetics of anti-HTLV-I antibody production (from 2 to 10 weeks a.t.) in a representative HTLV-I hu-SCID mouse (animal 598-5). The pattern at 2 and 4 weeks a.t. is identical to that of the HAM/TSP donor. Six weeks a.t. the reactivity tends to disappear, probably as a result of the death of most of the injected cells. **B:** Detection of viral HTLV-I tax sequences by PCR analysis in peripheral blood cells obtained between 2 and 10 weeks a.t. from the same HTLV-I hu-SCID mouse as in panel A. HTLV-I proviral sequences were present at 4 and 6 weeks a.t. Cells for PCR analysis and sera for Western blots were obtained at the same time every two weeks.

were found in brains and spinal cords of HTLV-I hu-SCID animals. Human lymphocytes were mainly found in the meninges and choroid plexuses. A small number of human PBL (either of B or T phenotype) were present in many of the mouse brains transplanted with PBL of HAM/TSP, but similar findings were observed in mice transplanted with PBL from multiple sclerosis patients as well as healthy subjects.

Seven HTLV-I hu-SCID animals developed a tumor that contained atypical human lymphoid cells. Five of the 7 (71%) tumors were PCR-positive for HTLV-I proviral sequences; all tumors were PCR-positive for EBV. Two of the 7 HTLV-I hu-SCID mice that developed a peritoneal lymphoid tumor showed metastatic invasion of the brain parenchyma. Immunocytochemical analysis identified the mononuclear cells in the peritoneal tumor (Fig. 4A,B) and corresponding brain (Fig. 4C,D) as predominantly B lymphocytes with 10–20% bearing a T lymphocyte marker.

DISCUSSION

HAM/TSP is a chronic progressive demyelinating myelopathy [Osame, 1990] of adulthood associated with HTLV-I infection. In contrast to ATL, HAM/TSP generally has several clinical and immunologic features that are distinct and include a shorter incubation time [Höllsberg and Hafler, 1993], higher levels of circulating anti-HTLV-I antibodies, polyclonal viral integration in lymphocytes [Greenberg et al., 1989] which spontaneously proliferate *in vitro* [Itoyama et al., 1988], and frequent oligoclonal distribution of antibody production in the cerebrospinal fluid [Grimaldi et al., 1988].

The pathogenesis of demyelination in HAM/TSP is poorly understood. Hypotheses include: a direct HTLV-I infection of oligodendrocytes; an infection of neural cells with a secondary cytotoxic attack (either cell- or cytokine-mediated) on HTLV-I-infected neural cells

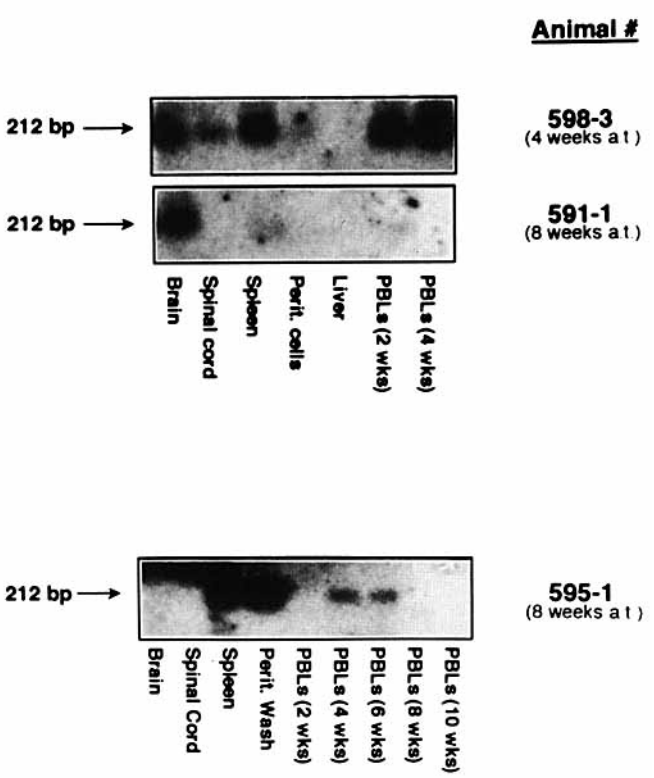

Fig. 3. Search for proviral HTLV-I LTR sequences by PCR analysis in 3 representative HTLV-I hu-SCID reconstituted with PBL from a single HAM/TSP donor. Animals were sacrified at 4 weeks (598-3) and 8 weeks a.t. (591-1 and 595-1). The brains from animals 598-3 and 591-1 are positive for HTLV-I sequences. Other neural and lymphoid tissues from animal 598-3 (but not 591-1) are also positive. The animal #595-1 gives an example of the kinetics of HTLV-I positive PBL in the blood (present at 4 and 6 weeks a.t.).

[Moore et al., 1989]; an autoimmune process resulting from HTLV-I infection and activation of autoreactive central nervous system (CNS)-specific HTLV-I-infected T-lymphocytes [Höllsberg and Hafler, 1993]. The availability of an animal model of HTLV-I infection would be valuable to clarify these issues.

Ishiguro et al. [1992] reported the development of clinical and pathological features similar to HAM/TSP in rats, 16 weeks after i.p. injection of neonates with an HTLV-I-infected human lymphoid cell line (10^7 MT-2 cells). Unlike HAM/TSP patients, no circulating antibody was detected in these animals. Kushida et al. [1993] found circulating anti-HTLV-I antibodies and HAM/TSP-like clinical features and spinal cord pathology in adult WKA rats, 20 to 27 months after intravenous injection of Ra-1 and MT-2 HTLV-I-infected cell lines. More recently, Feuer et al. [1993] transplanted PBL from ATL or HAM/TSP patients, as well as immortalized T-cell lines, in the peritoneum of SCID mice. Many of their animals became persistently infected with HTLV-I. T-cell lymphomas were found in 2 ATL-transplanted SCID mice, but not in any of the HAM/TSP transplanted SCID mice, suggesting that there is an important biological difference between cells infected by ATL- or HAM/TSP-associated HTLV-I.

By inoculating PBL from HAM/TSP patients into SCID mice we expected to reproduce neuropathologic features typical of the human disease. Unfortunately, evidence of CNS pathology (or clinical disease) was not found in a total of 50 HTLV-I hu-SCID animals over a 6 month period. Although we were not successful in producing an animal model of HAM/TSP during the brief window of time we tested SCID mice, we cannot exclude the possibility that a more prolonged period of time may be required for the development of clinical and pathological features typical of HAM/TSP in SCID mice. This may be a critical feature of disease induction considering that incubation periods of HAM/TSP in humans [Höllsberg and Hafler, 1993] and for HAM/TSP like-disease observed in rodents [Kushida et al., 1993] are rather lengthy.

However, other features of the human disease were reproduced in this model. We demonstrated the presence of HTLV-I genomic sequences by PCR in many of the HTLV-I hu-SCID mouse tissues for up to 14 weeks a.t.. This finding is similar to that of Kuroda et al. [1994]. It remains unclear whether the presence of HTLV-I genome in the HTLV-I hu-SCID mice merely reflects the continuous presence of HTLV-I positive human PBL. This, however, seems unlikely since human PBL, following their transplant into the SCID mouse, do not circulate long enough and in sufficient numbers to produce the strong viral genomic signals we recorded by PCR [Martino et al., 1993a]. An alternative explanation is the infection of mouse cells by HTLV-I. The latter possibility was not specifically addressed in our study.

Evidence was also found of circulating human anti-HTLV-I antibodies in HTLV-I hu-SCID mice for up to 14 weeks a.t.. The failure to detect pathological changes in the CNS of HTLV-I hu-SCID mice may then be due to the absence of a mouse HTLV-I cross-reacting autoantigen or to the irrelevance of humoral immunity in the pathogenesis of HAM/TSP. Other explanations include absence of a specific viral receptor in the mouse CNS; mismatch of MHC and cell-adhesion molecules; absence of an essential (viral?) co-factor.

Of interest was the finding that approximately 10% of HTLV-I hu-SCID animals developed intraperitoneal lymphomas of mixed B and T cell phenotype. Two animals had metastatic invasion of the CNS with these cells. Similar tumors have been seen in transplanted hu-SCID mice and are thought to result from inoculation of the mice with EBV-infected PBL [Custer et al., 1985]. In our animals, all tumors had evidence of EBV genomic sequences. We suspect that the brain metastases may reflect a predilection for the CNS by the lymphoma cells rather than a neural-specific tropism of HTLV-I-infected cells. The presence of HTLV-I sequences in 5 of the 7 tumors also suggests a possible interaction between HTLV-I and EBV.

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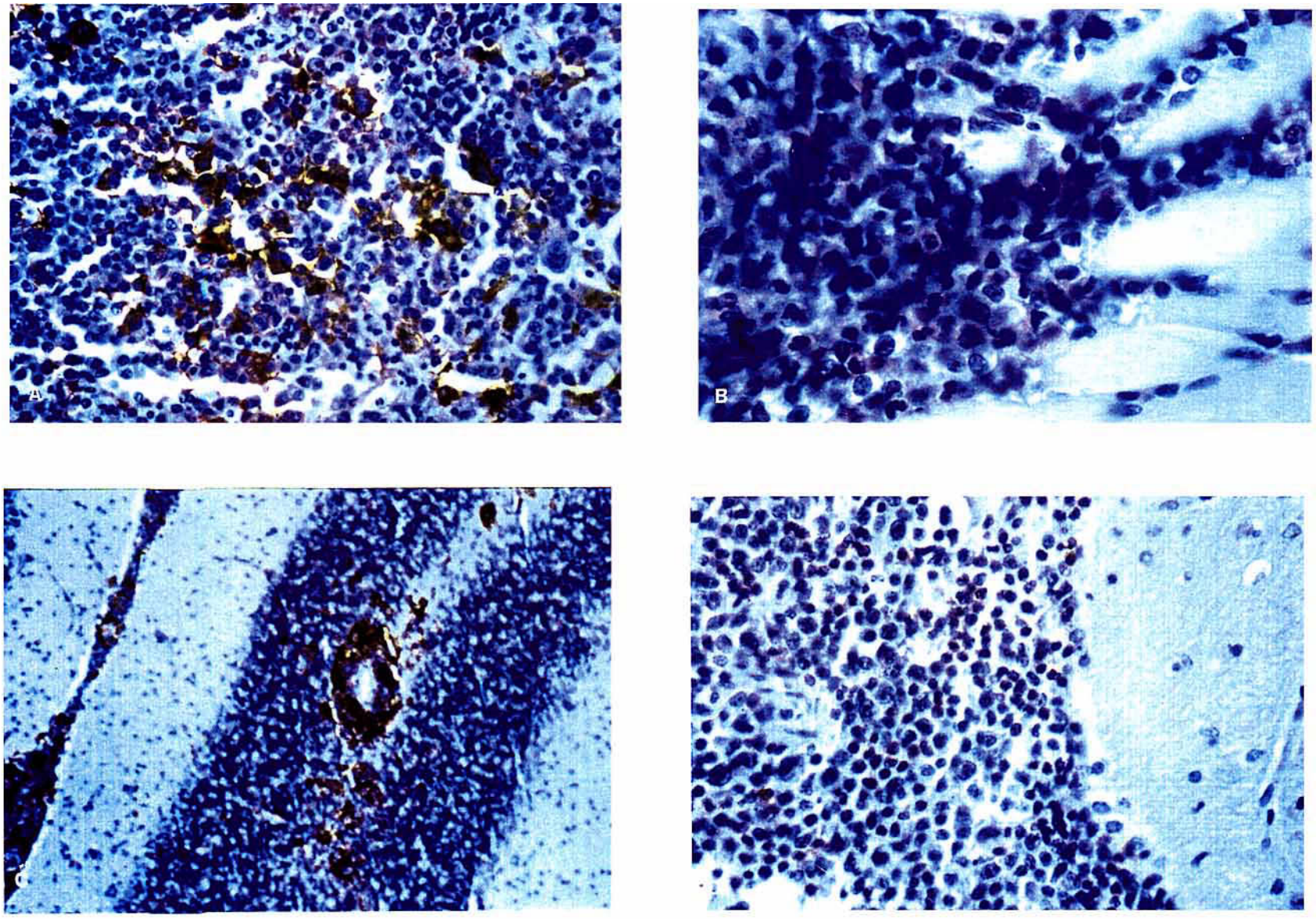

Fig. 4. Immunostaining of paraffin-embedded sections of a tumor found in the peritoneal cavity **(panels A,B)** and a corresponding cerebral metastasis **(panels C,D)** obtained from an HTLV-I hu-SCID mouse. Immunostaining was performed using anti-CD20 (B-cells; A,C) and anti-CD3 (T-cells; panels B and D) antibodies. The peritoneal lymphoid mass showed a prevalence of (A) human B-lymphocytes (×100) over (B) human T-lymphocytes (×125). The same relative amount of (C) human B-lymphocytes (×40) and (D) T-cells (×100) was also evident in the corresponding cerebral metastasis.

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